

### AMENDMENT TO THE CLAIMS

Please amend the claims as shown below. This listing of claims will replace all prior versions and listings of claims in the application. Deletions of text are indicated with ~~striketthrough~~ or double bracket [[xxx]]. Added text is shown by underline. Claim status is indicated as **withdrawn**, **currently amended**, **original**, **previously presented** or **cancelled**.

#### LISTING OF THE CLAIMS:

1. **(withdrawn)** A composition comprising an orthogonal leucyl-tRNA (leucyl-O-tRNA), wherein the leucyl O-tRNA comprises an anticodon loop comprising a CU(X)<sub>n</sub> XXXAA sequence, and comprises at least about a 25% suppression activity in presence of a cognate synthetase in response to a selector codon as compared to a control lacking the selector codon.
2. **(withdrawn)** The composition of claim 1, wherein the leucyl-O-tRNA comprises a stem region comprising matched base pairs and a conserved discriminator base at position 73 and wherein the selector codon is amber codon.
3. **(withdrawn)** The composition of claim 2, wherein the CU(X)<sub>n</sub> XXXAA sequence comprises CUCUAAA sequence and n=0.
4. **(withdrawn)** The composition of claim 2, wherein the leucyl-O-tRNA comprises a C:G base pair at position 3:70.
5. **(withdrawn)** The composition of claim 1, wherein the leucyl-O-tRNA comprises: a first pair selected from the group consisting of: U28:A42, G28:C42 and C28:G42; and, a second pair selected from the group consisting of: G:49:C65 or C49:G65; and, wherein the selector codon is a four-base codon.
6. **(withdrawn)** The composition of claim 5, wherein the CU(X)<sub>n</sub> XXXAA sequence comprises a CUUCCUAA sequence and n=1.
7. **(withdrawn)** The composition of claim 5, wherein the first pair is C28:G42 and the second pair is C49:G65.
8. **(withdrawn)** The composition of claim 1, wherein the CU(X)<sub>n</sub> XXXAA sequence comprises a CUUCAAA sequence and n=0, and wherein the selector codon is an opal codon.

9. **(withdrawn)** The composition of claim 1, wherein the leucyl-O-tRNA comprises or is encoded by a polynucleotide sequence as set forth in any one of SEQ ID NO.: 3, 6, 7 or 12, or a complementary polynucleotide sequence thereof.

10. **(withdrawn)** The composition of claim 1, wherein the leucyl-O-tRNA and cognate synthetase, or a conservative variant thereof, are at least 50% as effective at suppressing a selector codon as a leucyl O-tRNA of SEQ ID NO: 3, 6, 7 or 12, in combination with a cognate synthetase.

11. **(withdrawn)** The composition of claim 1, further comprising an orthogonal leucyl aminoacyl-tRNA synthetase (leucyl O-RS), wherein the leucyl O-RS preferentially aminoacylates the leucyl-O-tRNA with a selected amino acid.

12. **(withdrawn)** The composition of claim 11, wherein the leucyl O-RS, or a portion thereof, is encoded by a polynucleotide sequence as set forth in any one of SEQ ID NO.: 13 or 14, or a complementary polynucleotide sequence thereof.

13. **(withdrawn)** The composition of claim 11, wherein the leucyl O-RS comprises an amino acid sequence as set forth in any one of SEQ ID NO.: 15 or 16, or a conservative variation thereof.

14. **(withdrawn)** The composition of claim 1, wherein the leucyl-O-tRNA is derived from an archael tRNA.

15. **(withdrawn)** The composition of claim 1, wherein the leucyl-O-tRNA is derived from *Halobacterium sp NRC-1*.

16. **(withdrawn)** The composition of claim 1, further comprising a translation system.

17. **(currently amended)** A cell comprising a translation system, wherein the translation system comprises:

an orthogonal leucyl-tRNA (leucyl-O-tRNA) selected from:

(a) a leucyl-O-tRNA comprising a nucleotide sequence selected from SEQ ID NOS: 1, 2, 6, 7 and 12, 1, 2, 4-7 and 12, or

(b) a leucyl O-tRNA comprising (i) an anticodon loop comprising a CU(X)<sub>n</sub> XXXAA sequence, (ii) a first intramolecular base pair selected from U28:A42 and C28:G42, (iii) a second intramolecular base pair selected from G:49:C65 and C49:G65, and (iv) at least about 25% suppression activity in the presence of a cognate synthetase in response to a selector codon as compared to a

control lacking the selector codon, and where the selector codon is a four-base codon, or

(c) a leucyl O-tRNA comprising an anticodon loop comprising (i) a CUUCAA sequence, and (ii) at least about 25% suppression activity in the presence of a cognate synthetase in response to a selector codon as compared to a control lacking the selector codon, and where the selector codon is an opal codon;

an orthogonal aminoacyl-leucyl-tRNA synthetase (leucyl-O-RS) that comprises at least about 90% amino acid identity to a leucyl-O-RS of SEQ ID NO: 15 or 16; and, a first selected amino acid;

wherein the leucyl O-tRNA recognizes a first selector codon, and the leucyl O-RS preferentially aminoacylates the leucyl O-tRNA with the first selected amino acid.

**18. (previously presented)** The cell of claim 17, wherein the leucyl O-RS comprises an amino acid sequence as set forth in any one of SEQ ID NO.: 15, 16, and a conservative variation thereof.

**19. (currently amended)** The cell of claim 17, wherein the leucyl-O-tRNA and leucyl-O-RS are at least 50% as effective at suppressing the first selector codon as a leucyl O-tRNA of SEQ ID NO: 1, 2, 6, 7 and 12 ~~1, 2, 4-7 and 12~~ in combination with a leucyl O-RS comprising an amino acid sequence as set forth in SEQ ID NO.: 15 or 16.

**20. (previously presented)** The cell of claim 17, wherein the cell further comprises an additional different O-tRNA/O-RS pair and a second selected amino acid, wherein the additional O-tRNA recognizes a second selector codon and the additional O-RS preferentially aminoacylates the additional O-tRNA with the second selected amino acid.

**21. (previously presented)** The cell of claim 17, wherein the leucyl O-tRNA is derived from *Halobacterium sp NRC-1* and the leucyl O-RS is derived from *Methanobacterium thermoautotrophicum*.

**22. (original)** The cell of claim 17, wherein the cell is a eukaryotic cell.

**23. (original)** The cell of claim 17, wherein the cell is a non-eukaryotic cell.

**24. (original)** The cell of claim 23, wherein the non-eukaryotic cell is an *E. coli* cell.

**25. (previously presented)** The cell of claim 17, further comprising a nucleic acid that comprises a polynucleotide that encodes a polypeptide of interest, wherein the polynucleotide comprises or encodes at least one codon that is the first selector codon.

**26. (previously presented)** An *E. coli* cell comprising:

an orthogonal leucyl-tRNA (leucyl-O-tRNA) selected from:

- (a) a leucyl-O-tRNA comprising , wherein the leucyl-O-tRNA comprises a nucleotide sequence selected from SEQ ID NOS: 1, 2, 6, 7 and 12, 1, 2, 4-7 and 12, or
- (b) a leucyl O-tRNA comprising (i) an anticodon loop comprising a CU(X)<sub>n</sub> XXXAA sequence, (ii) a first intramolecular base pair selected from U28:A42 and C28:G42, (iii) a second intramolecular base pair selected from G:49:C65 and C49:G65, and (iv) at least about 25% suppression activity in the presence of a cognate synthetase in response to a selector codon as compared to a control lacking the selector codon, and where the selector codon is a four-base codon, or
- (c) a leucyl O-tRNA comprising an anticodon loop comprising (i) a CUUCAA sequence, and (ii) at least about 25% suppression activity in the presence of a cognate synthetase in response to a selector codon as compared to a control lacking the selector codon, and where the selector codon is an opal codon;

an orthogonal leucyl aminoacyl-tRNA synthetase (leucyl-O-RS), wherein the leucyl-O-RS preferentially aminoacylates the leucyl-O-tRNA with a selected amino acid; the selected amino acid; and,

a nucleic acid that comprises a polynucleotide that encodes a polypeptide of interest, wherein the polynucleotide comprises at least one selector codon that is recognized by the leucyl O-tRNA, and wherein the leucyl O-tRNA is derived from *Halobacterium sp NRC-1* and the leucyl O-RS comprises at least about 90% amino acid identity with a leucyl O-RS [[is]] derived from *Methanobacterium thermoautotrophicum*.

**27-61. (cancelled)**